primers. Based on their nucleotide sequences the 12 interacting clones were classified into 6 independent groups (see Table I).

TABLE I: Characterization of cDNA clones isolated by the yeast two hybrid screen.

Class	No. of clones	Identity	Mean RLU (Liquid assay)	Colour intensity (Filter assay)
1	6	Nedd4	$2.86x10^6$	++++
2	2	Htk	$1.86x10^5$	++
3	1	2.2412	$5.18x10^6$	++++
4	1	Proleosome	$3.88x10^2$	+/-
5	1	Solnatostatin	$1.45x10^3$	+/-
1	5	receptor		
6	1	L-arginine:glycine amidinotransferase	$8.61x10^2$	+/-

The 12 clones exhibiting activation of both the HIS 3 and lacZ reporter genes were divided into 6 groups by sequence analysis of their cDNA inserts. Results of ß-galactosidase activity assays performed using two methodologies are shown. The liquid culture-derived method (Gallacto-Light TROPIX) is more quantitative; results are given in mean relative light units (RLU) and are normalized for the protein content of the samples. Blue/white screening of the cDNA clones was also performed using a colony lift filter assay (Clontech). The intensity of blue colour development over approximately 2h is scored from +/- (very weak) to ++++ (strong).

Six clones were partial cDNAs corresponding to Nedd4, a multidomain protein containing a calcium-dependent phospholipid binding (CaLB) domain, four WW domains and a C-terminal region homologous to the E6-AP carboxyl-ternlinus (Kumar *et al. Biochem. Biophys. Res. Commun.* 185. 1155-1161, 1992; Sudol *et al J. Biol. Chem.* 270, 14733-14741, 1995; Huibregtse *et al Proc, Nail. Acad. Sci. USA* 92, 2563-2567, 1995). The latter is likely to confer E3 ubiquitin-protein ligase activity on Nedd4. The pACT2 clones isolated encoded the GalE domain together with the first 22 amino acids of the first WW domain.